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The medullary thick limb: Function and modulation of the single–effect multiplier

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The specialized transport properties of the thick ascending limb of Henle's loop (TALH) enable this segment of the nephron to dissociate salt and water absorption. Sodium chloride absorption by the cortical (cTALH) and medullary (mTALH) thick ascending limb accounts for urinary dilution, since each of these segments is virtually water–impermeable. Salt absorption by the mTALH also contributes to medullary interstitial hypertonicity, supplies the energy for the "single effect" of countercurrent multiplication, and hence modulates urinary concentrating mechanisms. The medullary (mTALH) and cortical (cTALH) portions of the TALH are clearly heterogeneous, and can be distinguished on the basis of morphological [1, 2], biochemical [3–6] and functional [7] differences.

This article will focus particularly on the transport functions of the medullary segment. The specific issues discussed will include the mechanism of NaCl absorption by the mTALH, the enhancement of this salt absorptive process by antidiuretic hormone (ADH), at least in certain mammalian species, and the major factors that appear to regulate or modulate both ADH-dependent and -independent NaCl transport processes. Sufficient information is currently available which indicates that these modulating factors provide feedback loops which sense interstitial osmolality and serve as a means of controlling the magnitude of interstitial hypertonicity, and hence concentrating power.

Some transport properties

In vivo micropuncture studies indicated that dilution of the urine emerging from the loop of Henle resulted from NaCl abstraction from the thick ascending limb of Henle [8]. In vitro microperfusion studies on the mTALH [9, 10] demonstrated four cardinal features of NaCl absorption by the medullary segment. First, Cl^- absorption occurred against a transepithelial electrochemical gradient. Second, net salt absorption resulted in a relatively large lumen–positive transepithelial voltage (V_e, mV) which was sensitive to the loop diuretic, furosemide, when applied from the luminal side. Third, both net Cl^- absorption and the transport–related transepithelial voltage depended on the activity of $(Na^+ + K^+)$-ATPase, present in large amounts along the basolateral membrane of this nephron segment [3]. Finally, the transepithelial ion conductance of the mTALH, calculated from passive tracer permeability measurements, indicates that this nephron segment is electrically "leaky" and that the shunt pathway is predominantly cation selective [7, 9, 10]. This latter feature is particularly unusual among epithelia with negligibly low water permeabilities.

Table 1 presents a summary of the important transport properties of the rabbit, rat and mouse mTALH that are relevant to the concentrating and diluting functions of this nephron segment. While it is clear that the mTALH segments from these species are quite similar with regard to the features discussed above, there are two significant differences between the rat (mouse) and rabbit. As these differences may play important roles in the concentrating process, it should also be noted that the maximal concentrating abilities of the rat and mouse are much greater than that of the rabbit.

The first important difference in the functional properties of the mTALH among these species is with regard to their urea permeabilities (P_{urea}). Knepper [11] has recently observed that the urea permeability of the rat mTALH is an order of magnitude greater than that of the rabbit (Table 1). Consequently, he has proposed that the P_{urea} of the TALH from the outer stripe of the rat outer medulla may be sufficient to permit the passive absorption of urea from this segment. This process not only would contribute to urinary dilution, by solute removal, but might also provide a pathway for medullary urea recycling, and thereby promote urinary concentration. Comparable data are not available on the urea permeability of the mTALH from mice.

The second major functional difference among these species is the much greater ADH-responsiveness of the adenylate cyclase system in rat and mouse mTALH segments [4–6]. The enhanced ability of ADH to stimulate this enzyme activity also appears to correlate with differences in the maximal concentrating abilities of the rat (mouse) and rabbit.

Site limiting mTALH water transport

The major functional property of the mTALH that permits this segment to dilute the urine is its negligible transepithelial water permeability. Since the ratio of junctional interspace to apical cell membrane surface area for the TALH is quite low [12], it seems reasonable to argue that the transcellular water permeability of this segment also be quite low. Recent qualitative and quantitative morphological observations in the mouse mTALH have indicated that the major barrier limiting transcel-

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Table 1. General characteristics of medullary thick ascending limbs

Species	ADH	P_f	$P_{D_{urea}}$	P_{Na}	P_{Cl}	$(Na^+ + K^+)$-ATPase	Adenylate cyclase
		$\mu m\ sec^{-1}$				*pmoles* $cm^{-1}\ sec^{-1}$	
Mouse	−	6-23		0.23	0.10	10	+ +
	+	6-23		0.25	0.12		
Rabbit	−	<5	<0.1	0.63	0.11	7	+ +
	+	<5					
Rat	−	≈0	0.7(IS), 1.4(OS)			13	−
	+	≈0					+

The data are from Refs. 3, 5, 6, 7, 10, 13.

lular water movement in this nephron segment resides at the apical (luminal) membrane [13].

Figure 1 shows the effects of perfusate and peritubular bath dilution on the tubular cell morphology of a single in vitro perfused mouse mTALH [13]. ADH was present throughout the experiment at a concentration that maximally stimulates NaCl absorption in this segment. During the initial period, isotonic Krebs–Ringer bicarbonate buffer (290 mOsm/liter; KRB) was present in both perfusing and bathing solutions. No change in the tubular cell morphology or estimated cell volume [13] was observed during about a six minute period after changing the perfusate to the dilute solution. However, very rapidly following bath dilution, tubular cells were seen bulging into the lumen (Fig. 1) and cell volume increased by 30%. These observations indicate that apical cell membranes of the mouse mTALH are virtually water-impermeant even in the presence of ADH. Therefore this membrane limits transepithelial water absorption in this region of the diluting segment.

General model of NaCl absorption

Analyses [7, 14–18] of the specific transport mechanisms involved in NaCl absorption by the mTALH, and the origin of the lumen-positive transepithelial voltage, may be integrated into the model shown in Figure 2. This model shares many similar features with those proposed for the rabbit cTALH [19] and non-mammalian diluting segments [20, 21]. The specific data in support of the model shown in Figure 2 have been reviewed elsewhere [22, 23]; we will therefore focus on some of the general features of the model.

Net Cl^- absorption by the mTALH involves a secondary active transport process in which luminal Cl^- entry into cells is mediated by an electroneutral Na^+:K^+:$2Cl^-$ co-transport process that is inhibited specifically by loop, or "high ceiling," diuretics. Recent studies assessing the ion dependence of Cl^- absorption by the in vitro perfused mouse mTALH indicate clearly that net Cl^- absorption requires both Na^+ and K^+ in luminal fluids [7, 15]. Measurements of tracer Cl^- uptake by either membrane vesicles or cells isolated from the medulla have [24–26], with few exceptions [27, 28], also demonstrated a dependence on both external sodium and potassium. In a recent report, Eveloff, Fong and Calamia [29] have indicated that a K^+ dependence for tracer Cl^- uptake in isolated rabbit mTALH cells could be demonstrated only after subjecting cells to hypertonic media. However, it is not clear whether this represents an intrinsic property of mTALH cells, or whether it is related to the method of preparation of the cells. Further evidence for the interdependence of Na^+, K^+ and Cl^- in the mTALH has been provided by Forbush and Palfrey [30], who demonstrated that the binding of (^{3}H)-bumetanide by apical membrane vesicles prepared from the outer medulla of dog kidneys was dependent on the presence of all three ions.

No direct measurements of the electrochemical gradients for co-transport of all three ions across the apical membrane of the mammalian mTALH have yet been made because of the difficulty in determining intracellular ion activity in these small cells. However, a favorable integrated electrochemical gradient for the electroneutral co-transport of Na^+:K^+:$2Cl^-$ units has been established for the amphibian diluting segment which possesses a similar mechanism for NaCl absorption [20, 31, 32]. At the present time, it seems reasonable to argue that the electrochemical gradient for Na^+ across the apical membrane is sufficient to drive the uphill movement of K^+ and Cl^- into cells. Thus apical Cl^- entry into cells depends ultimately on basolateral membrane $(Na^+ + K^+)$-ATPase. Consequently, ouabain addition to, or K^+ removal from, peritubular fluid inhibits salt transport in the mTALH [7, 10].

The exit of Cl^- from cells across the basolateral membrane is primarily conductive [15, 16, 18], although a small component of electroneutral KCl co-transport, which has been suggested for the rabbit cTALH [33], cannot be excluded entirely for the mTALH. The large intracellular negative voltage in the mouse mTALH [16] provides a portion of the driving force for electrical Cl^- exit. Based on the electrical properties of basolateral cell membranes, we have estimated [34] the intracellular Cl^- activities required to account for observed rates of Cl^- absorption: without ADH, the calculated Cl^- activity is 16 mM; and with ADH, 25 mM. Similar values for intracellular Cl^- activity have been measured in the rabbit cTALH without ADH [35].

An important characteristic of the mTALH, as well as all diluting segments, is the presence of a Ba^{++}-sensitive apical membrane K^+ conductance [15–17, 35]. The apical K^+ channels in the mouse mTALH share some of the characteristics of Ba^{++}/K^+ interactions observed in excitable tissue (such as, both voltage- and concentration-dependence and Ba^{++}/K^+ competitive effects [15–18]). Furthermore, these K^+ channels represent the sole conductive pathway identified to date in apical membranes of the mouse mTALH [15–18]. A role for this K^+ conductance in NaCl absorption by the mouse mTALH is indicated by the observation that luminal Ba^{++} can effectively abolish net Cl^- absorption [15–17, 34], presumably because luminal Ba^{++} depolarizes the cell and reduces conductive Cl^- exit across basolateral membranes [15, 16].

Recent estimates of the magnitude of the paracellular (and lateral intercellular space) conductance have provided a clearer

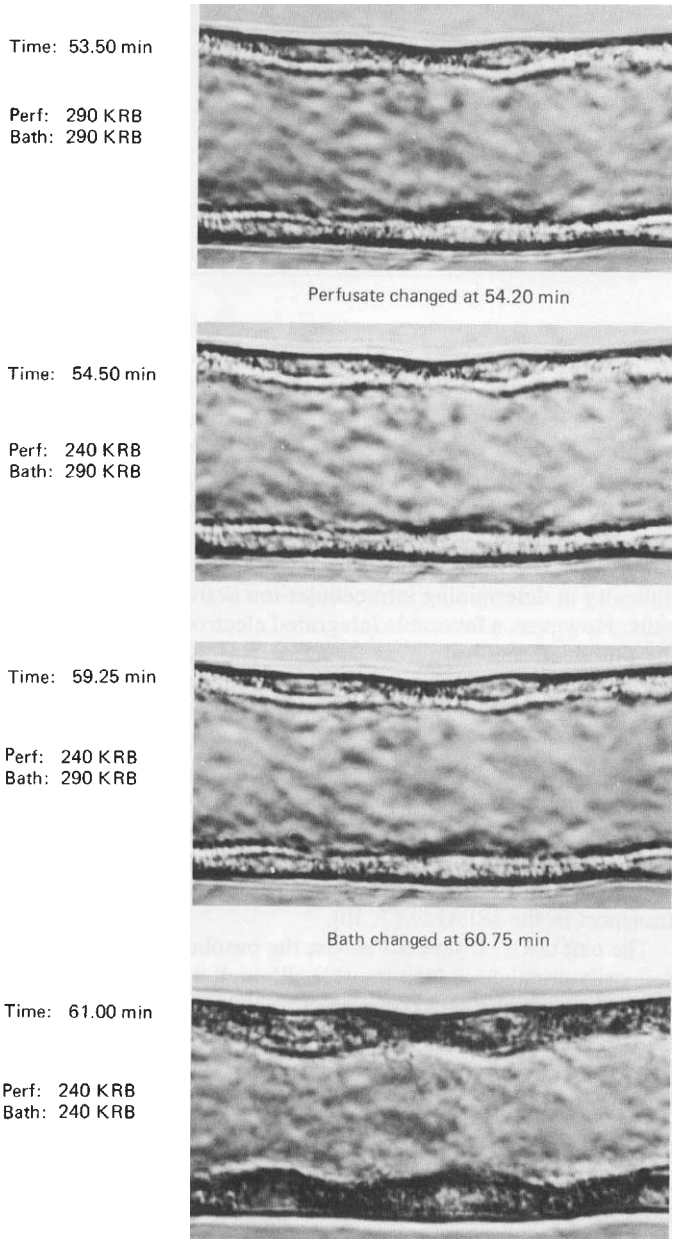

Fig. 1. *The rate limiting for transcellular water permeability in the mouse mTALH.* A single mouse mTALH segment was perfused in vitro at 37°C in the presence of bath ADH at 25 μU/nl. The perfusate and bath contained Krebs–Ringer's bicarbonate (KRB) media, 290 mOsm/KgH_2. The hypotonic KRB media were produced by reducing the NaCl concentration by 25 mM/liter: final osmolality was 240 mOsm/KgH_2O.

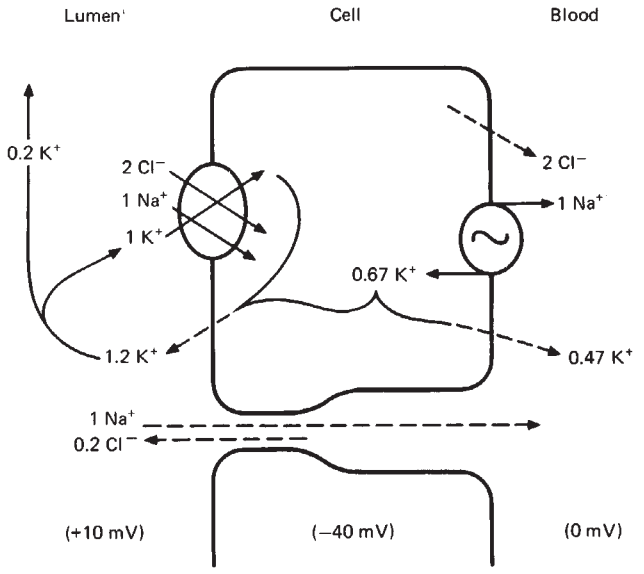

Fig. 2. *General model for NaCl absorption in the mouse mTALH.* From [15–17, 23].

picture of the important role played by both the apical K^+ conductance and the cation–selective paracellular pathway in transepithelial NaCl absorption by the mouse mTALH [17]. High concentrations of luminal Ba^{++} (20 mM, at zero luminal K^+) were used to block transcellular conductance, so that the residual transepithelial conductance represented, virtually exclusively, the paracellular pathway. Then a large reversed (lumen–to–bath, as opposed to normal luminal dilution) osmotic gradient was applied across the tubule by adding large concentrations of urea to luminal fluids. This maneuver produced a concentration–dependent increase in electrical conductance and a reduction in the Na^+/Cl^- permselectivity ratio of the paracellular pathway to free solution values. These functional changes were accompanied by blistering at the luminal surface similar to that found with apical hypertonicity effects in other water–tight epithelia [36].

These osmotic effects were referable to opening of the junctional complexes; thus the residual transepithelial conductance measured with luminal 20 mM Ba^{++} and with luminal 900 mM urea provided a minimal estimate of the lateral intercellular space resistance. This latter value was then used to calculate a maximal estimate of the concentration of NaCl in lateral interspace fluid that would occur during transepithelial NaCl absorption. The contribution of interspace NaCl accumulation to the transepithelial voltage could be determined from these data.

The results of these studies [17] indicate that the paracellular spaces are in virtual diffusion equilibrium with bulk solutions, so that transcellular, rather than paracellular, transport events must account for the origin of the transepithelial voltage. In other words, the differing ionic conductance properties of apical (principally K^+) and basolateral (primarily Cl^-) membranes appear to provide the critical pathways for the development of the lumen–positive transepithelial voltage. These observations, together with the close correspondence between the measured equivalent short circuit current and the sum of net Cl^- and K^+ transport in the mouse mTALH, indicate that Cl^- absorption is rheogenic, that is, associated with transcellular and paracellular current flows. According to this rheogenic model, the K^+ that enters the cell via the apical electroneutral salt co-transporter recycles, in large part, conductively across apical membranes, thus providing the apical component of the transepithelial current. In other words, K^+ recycling across apical membranes provides a means for maintaining electroneutral Na^+:K^+:2Cl^- entry across apical membranes, and blockade of the apical K^+

Table 2. Transport properties of mouse mTALH segments

V_e *mV*	J_{Cl}^{net} *pEq sec^{-1} cm^{-2}*	Shunt P_{Na}/P_{Cl}	Shunt G_{Na} *(mS cm^{-2})*	Shunt J_{Na}^{net} *(pEq sec^{-1} cm^{-2})*	J_{Cl}^{net}:shunt J_{Na}^{net} *ratio*
9.7 ± 0.5	10,950 ± 1180	3.0 ± 0.3	45.5 ± 4.7	4530 ± 550	2.4 ± 0.3

Data obtained from 13 mouse mTALH segments perfused in vitro [17] with ADH 10 μU/ml present in the peritubular bath throughout. Values represent mean ± SEM. The shunt values were calculated as indicated below while the tubules were perfused with 20 mm Ba^{++}/zero K^+ so that the transcellular conductance pathway was completely blocked. The paracellular Na^+ to K^+ permselectivity ratio (P_{Na}/P_{Cl}) was calculated from dilution voltage observed during reductions in bath NaCl concentration. The shunt $J_{Na}^{net} = 1/[F/G_s\ V_e\ (1 + 1/(P_{Na}/P_{Cl})]$, where G_s is the residual transepithelial conductance in the presence of 20 mm Ba^{++}/zero K^+ and F is the Faraday. The shunt $G_{Na} = 1/[G_s\ (1 + 1/P_{Na}/P_{Cl})]$. The net transcellular Cl^- absorption (J_{Cl}^{net}) was calculated as $J_e - J_K^{sec} = J_e - 1/[F/G_s\ V_e\ (1 + P_{Na}/P_{Cl})]$, where J_e is the equivalent electrical flux (I_{sc}/F).

conductance pathway by lumen Ba^{++} abolishes the transepithelial voltage because transepithelial currents are stopped.

One other prediction of the model shown in Figure 2 is also supported by these results. Since dilution of the urine by the mTALH occurs through the net absorption of equal quantities of Na^+ and Cl^-, a stoichiometry of $1Na^+$:$2Cl^-$ for the transcellular salt transport mechanism requires that one–half of the net Na^+ absorbed flows via the paracellular route. We have recently calculated the ratio of measured net Cl^- absorbed transcellularly to the net Na^+ that could be absorbed through the paracellular route [17]. The latter net Na^+ flux was estimated from the measured paracellular conductance, the electrical P_{Na}/P_{Cl} ratio and the observed magnitude of the transepithelial voltage. The results of these measurements are shown in Table 2. In accord with the prediction of the model shown in Figure 2, the magnitude of the transepithelial voltage in each tubule provides a driving force sufficient to transport a quantity of Na^+ through the cation–permselective pathway approximately equal to one–half the rate of net Cl^- absorption.

The mechanism of NaCl absorption in the mTALH shown in Figure 2 has at least two general consequences. First, rheogenic Cl^- absorption in the mTALH may reduce the energy expenditure required for transepithelial Na^+ transport. As discussed above, the combination of a lumen–positive transepithelial voltage and a high conductance, Na^+-selective shunt pathway permits the transport of about 50% of the total Na^+ absorbed through the paracellular route. Thus compared to epithelia that transport all the Na^+ absorbed through the transcellular route, the O_2 utilized to absorb a fixed quantity of Na^+ would be reduced to one–half. That is, assuming a fixed $3Na^+$:$2K^+$ stoichiometry for basolateral membrane ($Na^+ + K^+$)-ATPase, about 18 moles of Na^+ are transported transcellularly per mole of O_2 consumed; this may increase to approximately 36:1 for the mTALH [23].

Since the mTALH is generally thought to be in an in vivo environment with a low O_2 tension [37], the greater efficiency of its salt transport mechanism may be important for metabolic survival. In fact, recent observations in the isolated perfused rat kidney by Alcorn, Emslie and Boss and by Brezis et al [39] have demonstrated that the mTALH is uniquely sensitive to metabolic damage, due either to reduced O_2 delivery or to increases in transcellular ion transport. This phenomenon may account for the early loss of concentrating ability in certain forms of acute renal failure.

There may be one other advantage to the salt transport mechanism depicted in Figure 2. Schultz [40] has stressed the importance of homoregulatory processes in salt transporting epithelia which adjust the rate of Na^+ entry into cells to equal the rate of Na^+ exit, in order to avoid potentially lethal changes in cell volume. These processes are particularly important in epithelia like the mTALH where the rate of transepithelial salt transport can change rapidly. A mechanism that permits one–half of the net Na^+ movement to proceed via the paracellular route—and not affect cell volume—might be beneficial in the mTALH whose rate of NaCl absorption is about equal to, but whose cell volume is only about one–third that of the proximal tubule.

Table 3. Effect of ADH on NaCl and water transport in the mTALH

Species	ADH	G_e *mS cm^{-2}*	J_{NaCl} *pM sec^{-1} cm^{-2}*	V_e *mV*	References
Mouse	−	80	2600	5	[7, 15, 43, 44]
	+	100	10,800	11	[7, 15, 43, 44]
Rabbit	−		6400	3–7	[10, 44]
	+		unchanged	unchanged	[10]
Rat	−		3450[a]	2.5	[44]
	+		9070[a]	3.6	[44]

[a] Calculated from [44] assuming an inner tubule diameter of 20 μm.

Regulation of NaCl absorption

Stimulation of NaCl absorption by ADH

Wirz [41] first suggested that ADH might regulate countercurrent multiplication by augmenting the rate of NaCl abstracted from the ascending limb of Henle's loop. Added support for this possibility obtained by the recent observations of ADH-induced increases in adenylate cyclase [4–6] and protein kinase [42] activities in medullary thick ascending limbs of rabbit, rat and mouse.

Table 3 summarizes the effects of ADH on salt transport in the in vitro perfused mTALH segments of mouse, rat and rabbit. Hall and Varney [43] established that ADH increased the lumen–positive transepithelial voltage and the net rate of tracer Cl^- absorption in the mouse mTALH. Sasaki and Imai [44] and we [7, 14] confirmed these effects of ADH (and cyclic AMP) on salt transport in this species; this hormone effect was restricted to the medullary segment of the TALH [7, 14]. Moreover, the maximal effect of ADH on salt transport occurred at hormone concentrations found during antidiuretic states [7, 45]. Likewise, in homozygous Brattleboro rats, ADH (or 1-desamino-8-D-arginine vasopressin [dDAVP]) increases NaCl absorption in the mTALH of this species when assessed

by in vitro or by in vivo microperfusion of the loop of Henle [46, 47]. In contrast, no effect of pharmacologic concentrations of ADH (or cyclic AMP) could be demonstrated on salt transport in the rabbit mTALH [44] despite the significant, albeit variable, stimulation of adenylate cyclase by hormone in this species [4–6]. Insight into the varied effects of ADH on salt transport by the mTALH of these species may be gained by considering the interactions of ADH and osmolality. Since the osmotic environment of the mTALH varies with the state of water balance, such osmotic interactions may be quite important to hormone action in vivo. In renal medullary tissue, the effects of osmolality on both basal and ADH-stimulated adenylate cyclase activity are complex [48–52]. Increasing the NaCl concentration has a biphasic effect, with concentrations below 200 mM stimulating, but greater concentrations inhibiting, adenylate cyclase activity. However, urea consistently inhibits this enzyme at all concentrations above 50 mM. In contrast, DeRubertis and Craven [50] have observed vasopressin–stimulated cyclic AMP generation in renal medullary tissue increases in external osmolality up to 2000 mOsm/kg.

Torikai and Imai [52] have recently assessed the effects of osmolality on vasopressin–stimulated cyclic AMP generation in isolated fragments of rat mTALH. They find that hormone–stimulated cyclic AMP generation increases as a function of external NaCl, but not urea, concentrations. These results are consistent with the observations of Sasaki and Imai [44] that ADH stimulated NaCl transport in the isolated perfused rat mTALH only when the external fluids were hypertonic. Whether such osmolality effects can account for the apparent lack of hormone stimulating salt transport in the rabbit mTALH is unknown.

The issue of whether ADH can stimulate NaCl transport in the human mTALH is not clear. Chabardés and co-workers [4] were unable to detect any effect of ADH on cyclic AMP production in human mTALH segments isolated from pump–perfused kidneys. However, Alcorn, Emslie and Boss [38] have shown that, in isolated perfused rat kidneys, marked morphological changes occur in the mTALH within five minutes after starting perfusion. Given the unique sensitivity of the mTALH to hypoxic damage [39], it is possible that, in the pump–perfused human kidneys, hormone effects could be blunted.

ADH-prostaglandin interactions

The metabolism of renal medullary prostaglandins, and the interactions between ADH and these lipids, have been the subject of several recent reviews [53–55]. Prostaglandins are synthesized and rapidly inactivated in the renal medulla; their primary function is local. Prostaglandin E_2 is the major product of prostaglandin synthesis in the renal medulla, and is responsible for the major physiological effects of prostaglandins on water excretion.

At least two lines of argument suggest that PGE_2 may participate in a medullary feedback loop that modulates the rate of NaCl absorption by mTALH. First, PGE_2 has been shown to reduce directly the rate of net NaCl absorption in the mTALH, but not in the cTALH [56, 57]. Likewise, the possibility that PGE_2 inhibits in vivo NaCl absorption by the mTALH is supported by the micropuncture observations of an increase in $^{22}Na^+$ excretion in the urine when tracer $^{22}Na^+$ and PGE_2 were injected simultaneously into the late proximal tubule of the rat [58]; by the finding of a rise in NaCl content of the medulla when prostaglandin synthesis is inhibited [59]; and by alterations in loop chloride transport when inhibitors of prostaglandin synthesis are given [60, 61]. In contrast, Stokes [62] found that PGE_2, in micromolar concentrations, enhanced the ADH-unstimulated transepithelial voltage and net Cl^- absorption by the rabbit mTALH; however, an inhibiting effect of rabbit serum may have obscured a separate suppressive effect of PGE_2 on NaCl absorption [55, 57].

Second, there are specific interactions between PGE_2 and ADH in regulating NaCl absorption by the in vitro microperfused mouse mTALH [56, 63]. In this species, as opposed to the rabbit, micromolar concentrations of PGE_2 have no effect on NaCl absorption or the transepithelial voltage in the absence of ADH. However, in the presence of ADH, that is, when the rate of NaCl absorption is maximal, PGE_2 reduces the ADH-dependent component of NaCl transport in a dose–dependent fashion with half maximal inhibition occurring at about 10^{-10} M PGE_2. Likewise, PGE_2 inhibits ADH-dependent, but not basal, cellular AMP accumulation in isolated rat mTALH segments [64].

The PGE_2-ADH interactions in the mTALH are seemingly competitive, since supramaximal concentrations of ADH can reverse the inhibiting effect of PGE_2 on NaCl absorption [56, 63]. It now appears probable that PGE_2 inhibits the action of ADH by stimulating an inhibitory guanine nucleotide regulatory subunit of the adenylate cyclase system [63]. A similar mechanism may apply to PGE_2-ADH interactions on water permeability in the collecting duct [64]. A similar interaction likely occurs between PGE_2 and ADH in the rat.

It is unlikely that PGE_2 production by mTALH cells contributes to in vivo ADH-PGE_2 interactions, since cyclooxygenase activity is negligibly small in this nephron segment [66]. However, PGE_2 production may occur in other medullary components, including the medullary collecting duct and interstitial cells. PGE_2 synthesis by medullary/papillary interstitial cells can be modulated both by ADH and external solution osmolality [67]. These modulating factors appear to work by altering the calcium–dependent acyl hydrolase activity that regulates the availability of arachadonic acid for PGE_2 production by these cells.

The interaction among these modulating factors is complex. ADH can directly and acutely stimulate the synthesis of PGE_2 by interstitial cells, an effect that seems related primarily to the pressor, rather than the antidiuretic, activity of ADH [53, 54]. In contrast, during chronic ADH administration, antidiuretic activity may be important to an increase in cyclooxygenase activity [53, 54]. The role of ADH-PGE_2 interactions during physiological antidiuresis is unclear, since water deprivation in man [68] and animals [69, 70] may be associated with reductions in urinary PGE_2 excretion. Hypertonic NaCl also stimulates the release of PGE_2 from interstitial cells. Both this stimulation and that due to ADH can be reduced or abolished by hypertonic urea [67].

Osmolality effects on NaCl absorption

Medullary interstitial osmolality might regulate the rate of net NaCl absorption by the mTALH in at least two ways. First, increases in interstitial NaCl concentration produced by active NaCl absorption from the mTALH increase the driving force

for passive salt leakage from interstitium to luminal fluid via the paracellular pathway. Second, peritubular hypertonicity may down-regulate directly net salt absorption. Thus in the in vitro microperfused mouse mTALH, increases in the osmolality of peritubular bathing solutions with urea rapidly and reversibly inhibit the rate of active NaCl absorption [71]. This effect is not referable to the transtubular osmotic gradient, but rather to the magnitude of the increase in peritubular osmolality [71]. Since hypertonic urea does not affect either basal or ADH—stimulated cyclic AMP generation in the rat mTALH [52], it seems likely that processes beyond the generation of cyclic AMP participate in this urea-mediated inhibition. This view is supported by the observations that neither supramaximal concentrations of ADH nor dibutyryl-cyclic AMP are able to reverse the inhibition of NaCl transport mediated by hypertonic peritubular bath urea [71].

Other regulators of NaCl absorption

Adrenergic agonists. Adrenergic agonists can promote an effect similar to that produced by ADH in both man [72] and animals [73, 74]. Isoproterenol stimulates NaCl absorption by the mouse mTALH, and this effect can be inhibited by propranolol [75, 76]. Glucagon also augments the spontaneous transepithelial voltage in the in vitro microperfused mouse mTALH [76]. While these observations suggest that multiple agents may interact to regulate NaCl absorption by the mTALH [77], it should be noted that both isoproterenol and glucagon have affinity constants for action on the mTALH some three orders of magnitude higher than that for ADH, and that neither agent is additive to the effect of ADH [76].

Barajas and coworkers [78, 79] have demonstrated the presence of adrenergic nerve terminals along the basolateral membranes of the cTALH and mTALH in the rat. Furthermore, DiBona and Sawin [80] have shown that low frequency renal nerve stimulation increases the rate of salt absorption from the loop of Henle in both hydropenic and isotonic volume expanded rats; and, Bencsáth, Szénási and Takács [81] have observed reductions in loop NaCl absorption acutely following renal sympathectomy. These latter observations are consistent with the possibility that in vivo adrenergic nerve stimulation modulates salt transport by the TALH.

Medullary K^+ concentrations. Increased medullary K^+ concentrations occur by medullary K^+ recycling [82, 83]. The mTALH may play an important role in the recycling process [83, 84], and vasopressin may also stimulate medullary K^+ recycling by an action on the mTALH and on K^+ secretion in the distal tubule [15, 16, 85, 86]. The magnitude of medullary K^+ concentrations in the medulla are thought to be important in renal K^+ excretion [82–84]. Stokes [84], for example, observed a decrease in the rate of NaCl absorption from the in vitro microperfused mTALH following an increase in the external K^+ concentration from 5 to 25 mM, and suggested that the resultant increase in NaCl delivery to the distal convoluted tubule and collecting duct may enhance renal K^+ excretion. According to the model for salt absorption in the mTALH shown in Figure 2, the increased external K^+ (either luminal or peritubular) could depolarize mTALH cells via K^+ conductive pathways in apical or basolateral membranes, and thereby reduce the driving force for conductive Cl^- exit across the basolateral membrane. An acute effect of K^+ loading on loop NaCl absorption has also been observed in vivo [86].

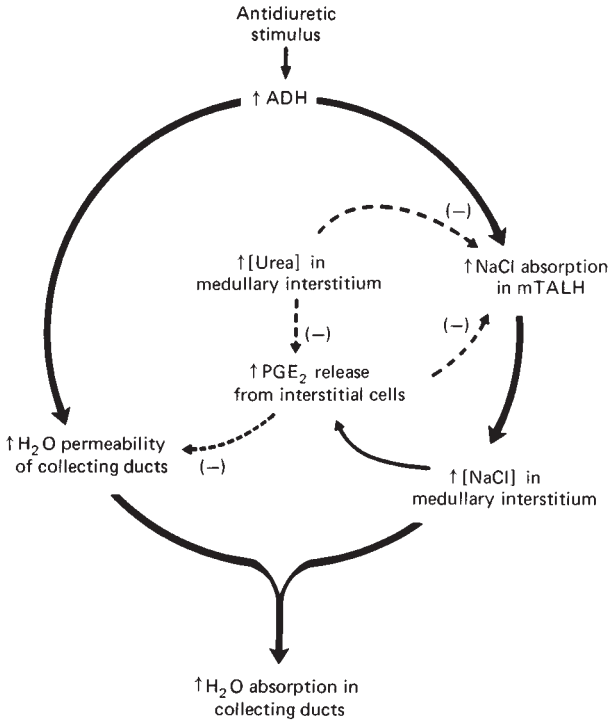

Fig. 3. *General model for regulation of urinary concentrating processes.* From [23].

Potassium deprivation is also associated with reductions in urinary concentrating ability. The micropuncture studies of Luke, Booker and Galla [87] in K^+ depleted rats suggest that a reduction in loop NaCl absorption could, at least in part, account for the impairment. The reduced loop NaCl absorption could result either from a reduction in ADH-sensitive adenylate cyclase activity or from a direct effect on low external K^+ on NaCl absorption by the TALH. Kim et al [88] have recently demonstrated that vasopressin-dependent adenylate cyclase activity, but not cell cyclic AMP accumulation, was reduced in K^+-depleted rats.

Calcium effects. Hypercalcemia reduces the ability to produce a maximally concentrated urine. This effect may be due, at least to some extent, to an inhibitory effect on ADH action [89, 90]. Since Ca^{++} may influence the adenylate cyclase–cyclic AMP system, it is possible that hypercalcemia may affect ADH-dependent cyclic AMP accumulation in the mTALH, a view consistant with the decreased medullary hypertonicity observed in the hypercalcemic state [89, 90]. Kusano et al [91] have provided evidence for an inhibitory effect of calcium on cyclic AMP accumulation in mTALH segments isolated from the rat. This effect appears to be due to a stimulation of phosphodiesterase activity. Thus, at least part of the hypercalcemic effect on urinary concentration may be by inhibition of ADH-stimulated NaCl absorption in the mTALH.

Model for integration of ADH, PGE_2 and osmolality effects

Figure 3 provides a model for integrating the effects of some modulators of urinary concentrating ability [23]. According to

the model shown in Figure 3, ADH-stimulated NaCl absorption by the mTALH is regulated by two negative feedback loops that "sense" the level of interstitial osmolality. In this manner, these feedback loops provide a means for controlling the magnitude of the driving force for water abstraction from collecting ducts, and thus urine concentration. During the early stage of antidiuresis, an ADH-mediated increase in NaCl absorption by the mTALH produces a rise in interstitial salt concentration [92–94]. The rise in interstitial NaCl concentration stimulates PGE_2 release from interstitial cells which, in turn, decreases the rate of ADH-dependent NaCl absorption. Since this ADH-PGE_2 interaction is concentration-dependent, alterations in ADH concentrations can influence the level of feedback. During antidiuresis, a rise in interstitial urea concentration decreases the hypertonic NaCl-mediated release of PGE_2 from medullary interstitial cells and directly inhibits salt absorption from the mTALH. It seems possible that this may provide a means of reducing the metabolic expenditure of energy by the mTALH in late antidiuresis, when high interstitial urea concentrations provide a major fraction of the driving force for water absorption from collecting ducts.

The ADH effect

It is now established that, in the mouse mTALH, ADH increases the rate of net salt absorption and the spontaneous transepithelial voltage V_e (Table 3). ADH also increases the transepithelial electrical conductance (G_e, mS cm^{-2}) exclusively because of an increase in transcellular electrical conductance (G_c, mS cm^{-2}); the hormone does not affect paracellular electrical conductance (G_s, mS cm^{-2}) [34]. Finally, ADH augments the net rate of K^+ secretion in mouse mTALH segments [15].

Some electrophysiologic considerations

In order to consider the mechanism for ADH action, it is pertinent to reiterate certain of the ionic conductance characteristics of these mTALH segments (Fig. 2). The apical membranes of the mouse mTALH are predominantly, and possibly exclusively, K^+ selective; these apical K^+ channels can be blocked by varying concentrations of luminal Ba^{++}, with zero luminal K^+ [15–18, 34]. The blockade of apical K^+ channels by Ba^{++} is readily reversible, either by removing luminal Ba^{++} and adding luminal 5 mM K^+; or, in the presence of luminal Ba^{++}, by raising luminal K^+ concentrations [15]. Cell impalement studies indicate that, with luminal Ba^{++}, the apical-to-basolateral membranes resistance ratio (R_a/R_b) rises about thirteenfold [16].

Finally, it is pertinent to note that with 20 mM luminal Ba^{++} there is virtually complete, but readily reversible, blockade of apical K^+ channels; and that the transcellular conductance G_c, when estimated in this manner, comprises approximately 40 to 50% of the total transepithelial conductance G_e [17, 34]. Consequently, in a given tubule segment one can measure: the transepithelial conductance G_e with luminal 5 mM K^+, zero Ba^{++}; the shunt conductance G_s (mS cm^{-2}) as the conductance insensitive to luminal 20 mM Ba^{++}, zero K^+; and the transcellular conductance G_c as ($G_e - G_s$) [17, 34].

As noted in Figure 2, basolateral membranes contain ($Na^+ + K^+$)-ATPase, a K^+ conductance, and a Cl^- conductance [15]. Net Cl^- absorption in the mTALH is virtually entirely conductive, and involves the basolateral Cl^- conductance [15, 17]. In agreement with this view, net Cl^- absorption may be abolished by applying Cl^- channel blocking agents to basolateral membranes [95].

An admittance mechanism for ADH

ADH may increase the net rate of salt absorption in the mTALH by way of a hormone-dependent increase in the functional number of Na^+:K^+:2Cl^- co-transport units in apical membranes [15, 16, 34]. Or put differently, ADH, acting through the second messenger cAMP, may exert an admittance effect on apical membranes. This postulate derives largely from an analysis of the interplay between ADH and furosemide in modulating transcellular ionic conductances. This argument, presented in detail elsewhere [34], may be summarized as follows.

Without luminal furosemide, ADH increases both the transcellular conductance G_c and the R_a/R_{bl} ratio; thus the ADH-dependent increase in G_c involves an increase in the conductance of basolateral membranes. Moreover, without furosemide, the hormone-dependent increase in V_e occurs because V_{bl}, the basolateral membrane voltage, is depolarized to a greater extent than V_a, the apical membrane voltage. However, when luminal furosemide is present, ADH produces no detectable increase in G_c; the R_a/R_{bl} ratio is about 0.5–0.6, and unaffected by ADH; and V_a and V_{bl} are hyperpolarized to the same extent, so that V_e is virtually zero with or without ADH.

Thus when furosemide is used to block hormone-dependent increases in the rate of apical Na^+:K^+:2Cl^- entry—which must attend hormone-dependent increases in the rate of net salt absorption—there is no ADH-dependent increase in G_c. Put differently, these data are consistent with the view that ADH increases the functional number of apical membrane Na^+:K^+:2Cl^- co-transport units, and that G_c rises because the attendant changes in intracellular ionic activities result in a depolarization of V_{bl} and an increase in basolateral conductance [15, 34]. More specifically, it is plausible to argue that the latter increases because the ADH-dependent increase in apical Na^+:K^+:2Cl^- units raises intracellular Cl^- activity, and thus depolarizes V_{bl}. According to this view, basolateral conductance rises because of a voltage-dependent rise in the Cl^- conductance of basolateral membranes [34].

In this regard, removal of bath Cl^- produces a striking reduction in G_c, and this reduction is considerably greater with ADH than without ADH; in other words, ADH increases basolateral Cl^- conductance [34]. However, this ADH-dependent increase in basal Cl^- conductance is blocked by luminal furosemide [34]. Moreover, by using the change in G_c, which occurs with bath Cl^- removal in combination with R_a/R_{bl} data, it has been possible to estimate the effects of ADH and furosemide on intracellular Cl^- activity [34]. Those calculations indicate that, without furosemide, ADH increases intracellular Cl^- activity from 16.3 mM to 25 mM; and that with furosemide, intracellular Cl^- is approximately 10.3 mM with or without ADH. These calculations are therefore congruent with the view that an ADH-dependent rise in intracellular Cl^- activity depolarizes V_{bl}, and produces a voltage-dependent increase in basolateral membrane conductance [34]. Thus, basolateral Cl^-

channels in the mTALH may have properties analogous to Goldman rectifiers.

It has also been proposed that ADH might increase the number of Cl^- channels in basolateral membranes [18]. In quantitative terms, this effect appears small with respect to the ADH effect on apical Na^+:K^+:$2Cl^-$ co-transport units.

Rather, the primary effect of ADH on mTALH segments, by way of the second messenger cAMP [14], is to increase net Cl^- absorption by increasing apical membrane Na^+:K^+2Cl^- admittance. Such an apical membrane admittance effect for ADH in the mTALH is analogous to that of ADH in other epithelia: in mammalian cortical collecting tubules and in amphibian epithelia, ADH or cAMP increases the functional number of apical membrane water [96–99], Na^+ [100] and K^+ channels [101]. In these latter ADH-sensitive epithelia, the hormone also increases the rate of fusion of sub-apical membrane vacuoles into apical intramembranous aggregates [102].

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